Facilitated Recovery of Protein Synthesis following Ischemia in the Brain with Induced Ischemic Tolerance

III. 7. Facilitated Recovery of Protein Synthesis following Ischemia in the Brain with Induced Ischemic Tolerance

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Introduction

Neurons in specific brain regions, such as those in the CA1 subfield of the hippocampus, are selectively vulnerable to ischemia.[1)] A 3- to 5-min period of ischemia damages the CA1 pyramidal neurons in gerbils.[2)] However, preconditioning of the brain with a 2-min period of sublethal ischemia followed by 1 to 7 days of reperfusion protects against neuronal damage following subsequent ischemia that normally kills the CA1 neurons.[2,3,4)] This phenomenon has been termed 'ischemic tolerance' and received attention because the elucidation of its mechanism may provide a clue for neuroprotection against ischemic brain damage.[2,3,4,5,6)] Thus far, heat shock proteins induced in the tolerance-acquired brain are suggested to play a role in the ischemic tolerance.[7,3,6)]

Protein synthesis in the brain is severely suppressed following ischemia.[8,9,10,11)] The suppressed protein synthesis recovers in ischemia-resistant regions, such as the CA3 subfiled of the hippocampus and the dentate gyrus, but never recovers in the vulnerable CA1 subfield. Thus, the recovery of protein synthesis may play a crucial role in the survival of ischemic neurons. Because the synthesis of the stress proteins mentioned above occurs during these periods of suppressed protein synthesis,[3,6)] postischemic alterations of protein synthesis in the brain with ischemic tolerance are of particular interest. We, therefore, studied the time course of protein synthesis in the brain with [^{14}C]leucine autoradiography in a gerbil model of ischemic tolerance.

Materials and Methods

Male adult Mongolian gerbils (Seiwa Expeimental Animals) were used. The animals were anesthetized with 2 % halothane in a mixture of 30 % oxygen and 70 % nitrous oxide. Both common carotid arteries were exposed and occluded with aneurysm clips for 2 min (ischemic preconditioning). Four days after the ischemic preconditioning or sham operation, the carotid arteries were again occluded for 3 min (second ischemia) and then reperfused. Body temperature during surgery and ischemia was maintained at 37 °C. Each group

consisted of 4-5 animals. Autoradiographic analysis of protein synthesis with [^{14}C]leucine was performed essentially as described previously.[8-11] L-[1-^{14}C]Leucine (New England Nuclear; specific activity 58 mCi/mmol, 10μCi each animal) was injected i. v. 4hr, 24 hr, and 48 hr after the second ischemia both with and without preconditioning. The animals were decapitated 35 min later and the brains were rapidly removed and frozen in powdered dry ice. Frozen sections, 20 μm thick, were cut on a cryostat and dried. Half of the sections were immersed in 5 % trichloroacetic acid (TCA) for 1 hr to remove nonincorporated free [^{14}C]leucine from the tissue. Both TCA-treated and non-treated sections were exposed to Kodak NMC-1 film for 4 weeks. The autoradiographs were then analysed with a computer-controlled image analysing system (IBAS image analyzer system, Zeiss) using [^{14}C]micro-scales (Amersham) exposed along with tissue sections. Radioactivity incorporated into brain proteins were calculated as nCi/g tissue and expressed as percent of normal control. Statistical significance was analysed with the analysis of variance followed by Duncan's multiple comparison test.

Results

In sham-operated animals, high protein synthesis was seen in the pyramidal cell layer of the CA1 and CA3 subfields of the hippocampus and the granule cell layer of the dentate gyrus. The neocortex and the striatum also showed high protein synthesis.

The protein synthesis decreased remarkably in the CA1 4 hr after 3 min of ischemia both with and without preconditining (Table 1). The protein synthesis in the CA1 never recovered in animals without preconditioning. However, in animals with preconditioning, it recovered in part in 3 of 8 hemispheres and fully recovered in 2 of 8 hemispheres after 24 hr. Full recovery was seen in all animals after 48 hr.

Reductions in protein synthesis were not observed in the CA3 and the dentate gyrus in both groups in this study except that the protein synthesis in the dentate gyrus was slightly reduced at 4 hr in some animals without preconditioning (Table 1).

In the superficial layer of the neocortex and the lateral striatum, the protein synthesis was reduced at 4 hr in animals without preconditioning but almost recovered after 24 hr. However, the decrease in the neocortex and the striatum was only exceptionally observed in the preconditioned animals.

Discussions

Earlier studies have shown that protein synthesis is initially globally suppressed after transient cerebral ischemia, but begins to recover in several hours.[8,10,11] The protein synthesis first recovers in ischemia-resistant regions, such as CA3 and dentate gyrus, but not in the vulnerable CA1 region. Thus, the idea that protein synthesis plays a causal role in the CA1 neuronal damage has been proposed.

During ischemia, protein synthesis is completely suppressed because of energy failure. Postischemic recovery of energy metabolism is rapid, but restoration of protein synthesis is much slower.[12] This postischemic suppression of general protein synthesis may be caused by a functional disturbance at the translational level probably due to the inactivation of polypeptide chain initiation factors and the disaggregation of polyribosomes.[12] Even after a sublethal 2-min period of ischemia in gerbils, protein synthesis in the CA1 is suppressed but recovers by 48 hr.[1] Therefore, protein synthesis probably returned to normal levels when the second ischemia was added in this study.

The comparison of the effects of ischemia on protein synthesis in gerbils with and without preconditioning indicates that ischemic preconditioning may protect against neuronal damage to CA1 neurons by facilitating recovery of protein synthesis. However, the preconditioning did not prevent the initial postischemic suppression of protein synthesis. Similar results have been reported using [^{3}H]valine,[13] but the quantitative data of this study showed that facilitated recovery was seen in many regions of the brain including the neocortex and the striatum. Histological neuronal damage is not observed in these regions following 3-min ischemia.[2] Therefore, only protein synthesis, which is a sensitive marker of ischemic injury, could detect sublethal injury in these regions. Thus, acquisition of ischemic tolerance may be obtained in various regions of the brain.

The observation also demonstrates that the preconditioning may not ameliorate the initial injury by ischemia. This finding may be in accordance with our previous observations that ischemic preconditioning did not alter the amount of excitatory and inhibitory amino acids released during the second ischemia as shown by intracerebral microdialysis,[14,15] and that the postischemic alterations in ubiquitin immunoreactivity were very similar to those of protein synthesis.[16]

The present result, therefore, suggests that the development of ischemic tolerance requires an additional protective factor, which alone or in combination with the recovery of protein synthesis is responsible for the induction of tolerance. An alternative explanation may be activation of heat shock protein (HSP) genes by ischemia.[7,3,5,6] The fact that the induction of HSPs takes place during the periods of general protein synthesis suppression may support the notion that the HSPs are preferentially produced to protect against ischemic damage. The protective effects of the HSPs against a wide range of harmful stresses have been well documented.[17-19]

In summary, this study shows that the mechanism of postischemic neuronal protection in ischemic tolerance is related to the recovery of protein synthesis. The present findings that the suppression of protein synthesis was not ameliorated during the early postischemic reperfusion periods suggest that factors other than protein synthesis may be of greater importance for the manifestation of ischemic tolerance. The nature of the factor that responds to ischemic preconditioning is of considerable importance for the understanding of the mechanism of ischemic tolerance and deserves further study.

References

1) Araki T., Kato H. and Kogure K., Acta Neurol. Scand., **80** (1989) 548.
2) Kato H., et al., Brain Res., **553** (1991) 238.
3) Kirino T., Tsujita Y. and Tamura A., J. Cereb. Blood Flow Metab., **11** (1991) 299.
4) Kitagawa K., et al., Brain Res., **528** (1990) 21.
5) Liu Y., et al., Brain Res., **586** (1992) 121.
6) Liu Y., et al., Neuroscience, **56** (1993) 921.
7) Kato H., et al., Brain Res., **634** (1994) 249.
8) Araki T., et al., Acta Neuropathol., **79** (1990) 501.
9) Dienel G.A., Pulsinelli W.A., and Duffy T.E., J. Neurochem., **35** (1980) 1216.
10) Thilmann R., et al., Acta Neuropathol., **71** (1986) 88.
11) Widmann R., et al., J. Neurochem., **56** (1991) 789.
12) Hossmann K.-A., Prog. Brain Res., **96** (1993) 161.
13) Nakagomi T., Acta Neuropathol., **86** (1993) 10.
14) Kato H., Nakata N. and Kogure K., J. Cereb. Blood Flow Metab., **13** (Suppl. 1) (1993) S788.
15) Nakata N., et al., Neurosci. Lett., **138** (1992) 86.
16) Kato H., et al., Brain Res., **619** (1993) 339.
17) Landry J., et al., Cancer Res., **42** (1982) 2457.
18) Landry J., et al., J. Cell Biol., **109** (1989) 7.
19) Lindquist S., Ann. Rev. Biochem., **55** (1986) 1151.

Table 1. Postischemic alterations in protein synthesis (% changes compared with control) in brain regions in animals with and without ischemic tolerance

	4 hr		1 day		2 days	
	tolerance(-)	tolerance(+)	tolerance(-)	tolerance(+)	tolerance(-)	tolerance(+)
Frontal cortex (superficial layer)	55±8.5**	92±17.5##	85±5.3	95±24.0	109±12.7	108±11.5
Lateral striatum	70±20.1	106±15.0#	86±25.3	115±33.8	114±19.2	121±18.3
Hippocampus CA1 (pyramidal layer)	29±11.9**	25±7.8**	27±8.8**	60±12.4**##	33±5.7**	107±12.3##
Hippocampus CA3 (pyramidal layer)	97±20.8	106±8.5	94±19.6	102±25.4	117±16.1	112±5.9
Dentate gyrus (granule cell layer)	89±21.1	117±19.7#	118±22.5	103±11.1	120±13.1	118±16.4
Ventral thalamus	113±12.8	115±6.0	90±21.5	94±20.5	128±10.1*	117±8.6

Values are expressed as means ± SD. n=4-5.
*p<0.05, **p<0.01 compared with normal control,
#p<0.05, ##p<0.01 compared with animals without tolerance (Duncan's multiple comparison test)